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Structural and functional aspects of carboxysomes of thiobacillus neapolitanus.

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Document Version

Publisher's PDF, also known as Version of record

Publication date:

1986

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Holthuijzen, Y. A. (1986). Structural and functional aspects of carboxysomes of thiobacillus neapolitanus. s.n.

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SUMMARY

Carboxysomes are regularly shaped, hexagonal inclusion bodies, 120 nm in diameter which are surrounded by a 3-4 nm thick shell. They are found in many autotrophic prokaryotes. These inclusion bodies have attracted considerable interest since Shively noticed that they contain the CO₂ fixing enzyme D-ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). Autotrophic organisms which are dependent on this enzyme for their growth contain RuBisCO soluble in the cytoplasm and, in some cases, packed in the carboxysomes. Why, sometimes, a part of the total RuBisCO is packed inside the carboxysomes is an intriguing question, which yet has to be answered.

The aim of this study is to characterize the composition and structure of the carboxysomes of the chemolithotrophic Thiobacillus neapolitanus in order to obtain information about their function. T. neapolitanus is chosen for a number of reasons, the most important being that 1) much is known on the general physiology of this organism, 2) extensive knowledge is available on its RuBisCO, 3) the organism can easily be grown under well controlled conditions whereby high or low numbers of carboxysomes are produced, and 4) the hypothesis that carboxysomes of this organism are functional calvinosomes has to be tested.

In Chapter II an isolation procedure is described which results in a carboxysome preparation free from whole cells, protoplasts and cell fragments. The purified carboxysomes consists of 8 proteins and at the most 13 polypeptides. Two proteins appear to be most abundant in the gels: RuBisCO and a glycoprotein with approximately the same molecular weight (56,000 daltons) as the large subunit of RuBisCO. The two proteins make up more than 60 % of the total protein of the carboxysome and are found in a ratio RuBisCO/glycoprotein of 55 to 45. The shell of the carboxysomes consists mainly of glycoproteins. Apart from the major glycoprotein of 56,000 daltons, the shell contains three other glycoproteins with molecular weights of 120,000, 85,000 and 10,000 daltons in minor quantities.

RuBisCO is the only enzyme which could be found in the carboxysomes. The activity in carboxysomes is 2.1-2.4 μ moles CO₂ fixed/min.(mg protein) (Chapter II,V) and this activity does not change upon dilution or after 1 time 30 s sonication (Chapter V). Carboxysomes which are subjected to longer periods of sonication show gaps from which 10 nm particles are released. Separation of the shell and contents of the carboxysomes reveals that the particles consist of the large subunit of RuBisCO. The shell proteins and the small subunit of RuBisCO are always found together which indicates that the RuBisCO molecules are connected to the shell proteins via the small subunit. After 3 times of 30 s sonication about half of the carboxysomes are disrupted

and the activity decreases to 160 nmoles CO_2 fixed/min.(mg protein). The decreased activity of RuBisCO is possibly caused by a dissociation of the enzyme in large and small subunits. This dissociation probably precedes the disintegration of the carboxysomes, which is complete after 20 times 30 s sonication (Chapter V).

Isolated carboxysomes are always found together with double-stranded DNA. This DNA is not an integral part of the carboxysomes since treatment with DNase I causes a degradation of the DNA, but has no effect on the carboxysomes. The DNA which is attached to the carboxysomes is heterogeneous in size. The electrophoretic mobility, restriction enzyme digestion, and hybridization experiments indicate that this DNA is chromosomal DNA (Chapter IV)

The structure of carboxysomes have been studied electronmicroscopically (Chapter III). The outer part, the shell, is examined in freeze dried and fixed carboxysomes. Stereomicrographs from these carboxysomes reveal their three-dimensional structure which appears to be a pentagonal dodecahedron. This means that carboxysomes possess twelve similar pentameric planes. This conclusion is consistent with the finding that carboxysomes appear always as hexagonal bodies with dissimilar edges. Inside the bodies a regular arrangement of the RuBisCO molecules is observed. Since the RuBisCO particles appear mostly in rows and sometimes as coiled forms (helical lines) it is assumed that the RuBisCO molecules form one layer against the inside of the shell.

Thiobacilli can use carbon dioxide as sole carbon source and since the carboxysomes are clearly involved in the CO_2 fixation it seems interesting to study the mechanism of uptake of CO_2 in cells during CO_2 and thiosulphate limitation. In Chapter VI the results of this study have been presented. Uptake of inorganic carbon (CO_2 , HCO_3^-) in whole cells of T. neapolitanus is an energy-dependent transport process, since uptake is fully inhibited by uncouplers. In thiosulphate-limited cells accumulation ratios of 500- to 800-fold are measured. In CO_2 -limited cells these ratios are significantly higher (1000- to 1500-fold). The same results are obtained from CO_2 fixation: high levels of fixed inorganic carbon are found in CO_2 -limited cells and relatively low levels in the thiosulphate-limited cells. These results can be expected since CO_2 -limited cells contain five-fold higher RuBisCO activity than cells grown under thiosulphate limitation. Studies involving direct measurements of the proton motive force, and the use of suitable inhibitors demonstrate that the proton motive force and especially one of its components, the electrical potential, is responsible for the uptake of inorganic carbon. However, uptake of inorganic carbon is never observed without electron transfer. To transport inorganic carbon, active respiration is an additional requirement. The carbon species, CO_2 or HCO_3^- which is

transported, is investigated by measurement of the K_m values for transport of these species at different pH values. The data show that a relatively constant affinity can be found for CO_2 but not for HCO_3^- at the different pH values which indicating that CO_2 is the species transported.

In Chapter VII the data of our studies described in Chapters II-VI are discussed and a model of a carboxysome is presented. Our data demonstrate that the carboxysomes are active in CO_2 fixation. However, it is not clear why RuBisCO molecules are packed in carboxysomes. Therefore several possibilities for the existence of carboxysomes are proposed: a) they prevent the entry of oxygen and subsequently the oxygenase reaction of RuBisCO, b) they protect RuBisCO against proteolytic degradation and c) they prevent an increase in osmolarity in the cytoplasm during CO_2 limitation when the concentration of RuBisCO increases considerably.